



Further Observations on *Meloidogyne indica* Whitehead, 1968 from India

Matiyar Rahaman Khan^{1,*}, Sabayasachi Pal², Amit Singh²,
Ashok Dhananjaybhai Patel³, Bhagubhai Ambaram Patel³,
Tushar Manohar Ghule² and Victor Phani¹

¹Division of Nematology, Indian Agricultural Research Institute, Delhi, India

²Department of Agricultural Entomology, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal, India

³Department of Nematology, Anand Agricultural University, Anand, Gujarat, India

ABSTRACT

Infestation of citrus root knot nematode (*Meloidogyne indica*) on the roots of citrus and Bt-cotton was found in Gujarat of India. The detection of *M. indica* and its diagnostic symptoms on some hosts (citrus and Bt-cotton) was recorded and the nematode species was authentically identified as *Meloidogyne indica* Whitehead, 1968 which was first time described on citrus from Delhi, India. The species is further characterized based on detailed morphological, morphometric descriptions and beta-esterase phenotyping. The present study elucidated information on *M. indica* for its easy detection, diagnosis, dimension of damages on citrus, and Bt-cotton under Indian conditions.

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Authors' Contribution

MRK conceived and designed the idea and wrote the manuscript. SP, TMG, AS and MRK performed the lab work. MRK and ADP collected samples. VP helped in compilation of data and preparation of manuscript. SP and MRK analysed the data.

Key words

Morphology, Symptoms, *Meloidogyne indica*, Citrus, Bt-cotton.

INTRODUCTION

Citrus spp. (family Rutaceae) is one of the most preferred fruits all over the world known for its flavor, rich source of vitamin C, minerals and dietary fiber (non-starch polysaccharides) that are essential for normal growth and development. Several root-knot nematodes have been reported to parasitize citrus in Asian countries, viz., *Meloidogyne citri*, *M. donghaiensis*, *M. fujianensis*, *M. mingnanica*, *M. jianyangensis*, *M. kongi*, *M. incognita* and *M. indica* (Davis and Venette, 2004). In India, Gujarat state shares about 4.0% of total citrus production of the country (Anonymous, 2014) and two most popular cultivars viz., Ranpur lime and kagzi lime are grown widely in the state. The occurrence of *M. indica* on kagzi lime (*Citrus aurantifolia*) was reported from north Gujarat (Patel et al., 1999). The citrus orchards were found to have a severe infestation of root-knot nematodes. Bt-cotton is becoming popular among the conventional citrus growers and the popular crop sequences in Gujarat are cotton (summer)-mustard/wheat (winter) or castor (winter)-cotton (summer). Bt-cotton, in this part of country, was also found to have problems of root-knot nematode infestation. Preliminary observation on the root-knot nematode infecting citrus and Bt-cotton (Khan et al., 2014) revealed resemblance to *Meloidogyne indica* Whitehead, 1968, first time reported

and described on citrus from Delhi, India (Whitehead, 1968). Since description, *M. indica* is not known much from India and it was not further characterized from elsewhere. Currently the information available on *M. indica* is thought inadequate for better understanding the species. Therefore, the present study was focused on further characterization of the species based on morphological, morphometric and biochemical information.

MATERIALS AND METHODS

Nematode populations and processing of samples

Samples of nematode infested roots of citrus (*Citrus aurantifolia* cv. kagzi lime) and Bt-cotton plants along with rhizospheric soil of the infested plants were collected from Kharva village of Mehsana district, Gujarat, India; located at N23°31'70" and E72°27'63". The fields of Bt-cotton were in fact previously infested with root knot nematodes in citrus. Other crops like castor, *Capsicum* sp. and brinjal were also sampled. The soil type in the sampled areas is primarily sandy to sandy loam with neutral pH (6.5-7.5); soil organic carbon is medium with low phosphorus and high potassium. The weather conditions are primarily characterized by extreme summer (March to June) when the temperature goes up to 46°C, and cool winter (November to February) when it is as low as 9°C, with total annual rainfall about 800-900 mm.

The root samples were stained by NaOCl-acid fuchsin method (Byrd et al., 1983) and a part of the collected root samples were cut into small pieces for extraction of second

* Corresponding author: drmrkhanbckv@gmail.com

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stage juveniles (J2s) and males by modified Baermann's technique (Whitehead and Hemming, 1965). Extraction of J2s and males from soil was also pursued by the same technique. Egg masses of root-knot nematodes were collected with the help of forceps from the stained root sample and eggs from egg masses were released by teasing them on a glass slide with a needle under stereozoom trinocular microscope (Carl Zeiss: Stemi 2000 C) at 40X magnification.

Host test

Nematode infested roots and soil from citrus field was mixed with sterile soil and distributed in 10 earthen pots (10 cm dia). At least three pots for each crop were planted with three week-old seedlings of tomato (cv Rutgers), brinjal (cv local), and citrus (kazgi lime, cv local). An additional pot with sterile soil was maintained for each crop as control check. Sixty days after planting, individual plant was uprooted and observed for nematode infection and reproduction.

Morphological and biochemical studies

Nematode specimens for morphological observations were killed by hot-water bath, then fixed in 4% formalin solution and stored with proper labeling. Subsequently the specimens were processed by glycerol-ethanol method (Seinhorst, 1959) for light microscopic studies. The processed specimens were then mounted in anhydrous glycerine on glass slides. The morphological characters for *Meloidogyne* as described by Jepson (1987) and Karssen (2002) were included for in depth identification and characterization of the species. The de-Man ratios and other values were determined from measurements taken with the help of an ocular micrometer (Hooper, 1986). Photomicrographs of males, females and juveniles were taken with a Color Digital Camera (Cannon Power Shot, 20IS) attached to a compound microscope (ZEISS-AXIOSKOP 40, Germany). The beta-esterase phenotype of the nematode population was analyzed as per protocol of Esbenshade and Triantaphyllou (1985) and its modification (Khan *et al.*, 2017).

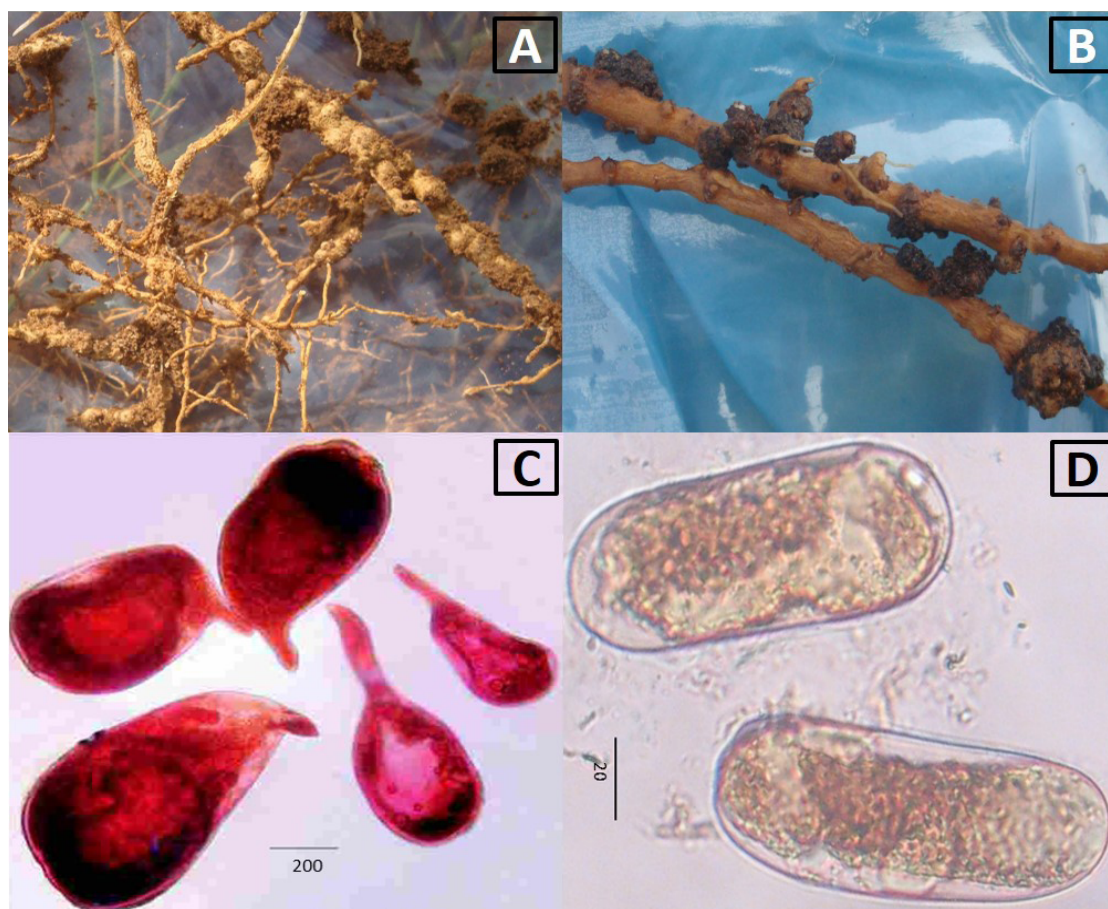


Fig. 1. Symptoms (A, B) induced by *Meloidogyne indica* in different hosts. Galls on Citrus root (A), Bt-cotton root (B), Adult female (C) and Egg (D). Scale –bar in μm .

RESULTS

Diagnostic symptoms and identification

Citrus root-knot nematode (*M. indica*) is on firm footing on citrus and Bt-cotton growing areas of Mehsana district of Gujarat, India. Nematode infected citrus plants exhibited symptoms almost similar to dieback or decline of older plants. The citrus plants typically exhibited sick appearance, yellowing of leaves, drying of twigs and yielding no fruits. Each plant (age 3-5 year-old) with such symptoms confirmed the infestation of root knot nematode. Inspection of citrus along with castor, capsicum, brinjal grown on adjoining fields revealed occurrence of *M. indica* infestation on citrus (Fig. 1A) only. The castor crop grown in the nearby field showed no infection of root-knot nematodes. However, galled root of brinjal and capsicum showed the presence of *M. incognita* (Kofoid and White)

Chitwood, 1949. Bt-cotton plants were found to be badly damaged by the infestation (Fig. 1B) of *M. indica*.

The observation on crop hosts viz., tomato (cv Rutgers), brinjal (cv local), and citrus (kazgi lime, cv local) grown on the root-knot nematode-infested soil, after three months (January-March, 2011) revealed infection of *M. incognita* on tomato and brinjal, while citrus root showed typical infestation of *M. indica*. The identification of species of *Meloidogyne* was confirmed based on morphological observations and beta-esterase phenotyping analysis.

Morphology and morphometrics of M. indica

The detailed morphology of adult female, male and second stage juvenile of *M. indica* infecting Citrus and Bt-cotton and their morphometrics were studied for characterization and confirmation of species.

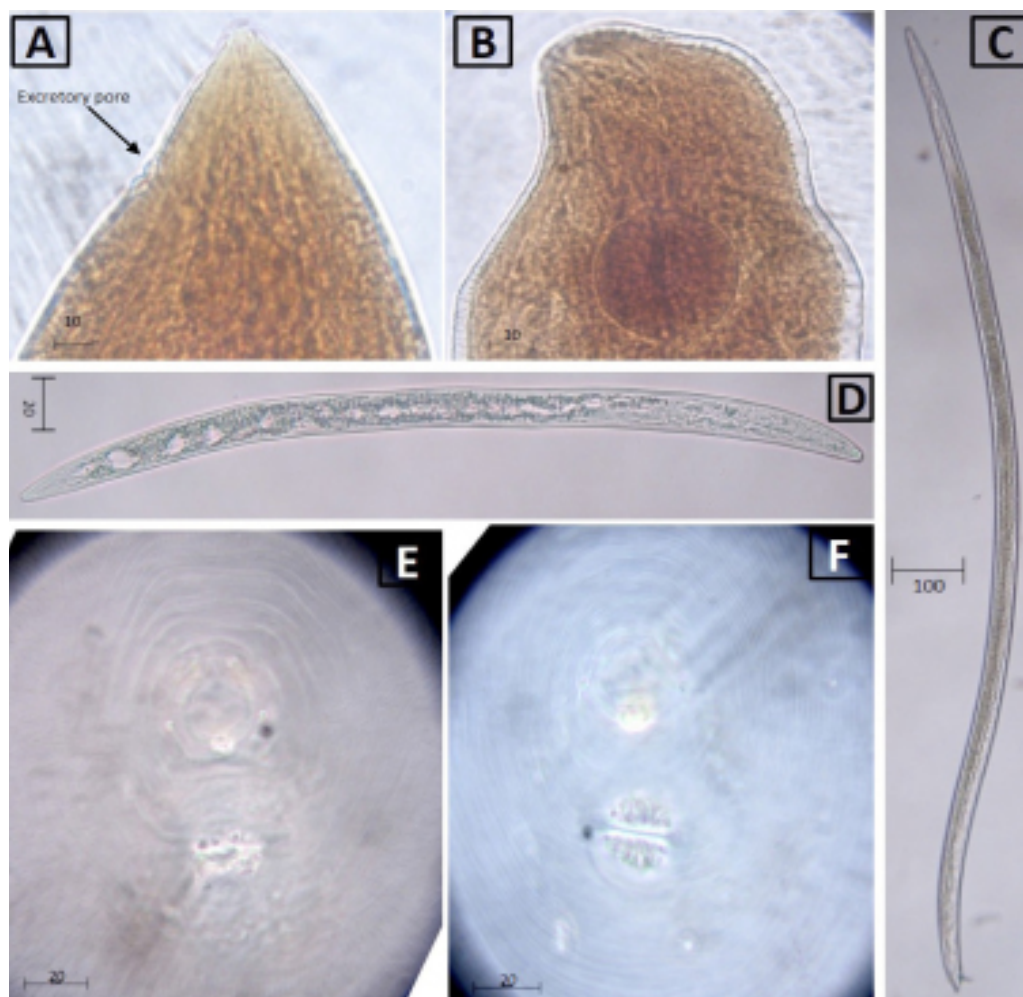


Fig. 2. *Meloidogyne indica*. A and B, Variations in female head; C, Second stage juvenile; D, Adult male; Perineal pattern: Mehsana (citrus) population (E); Mehsana (Bt-cotton) population (F). Scale –bar in μm .

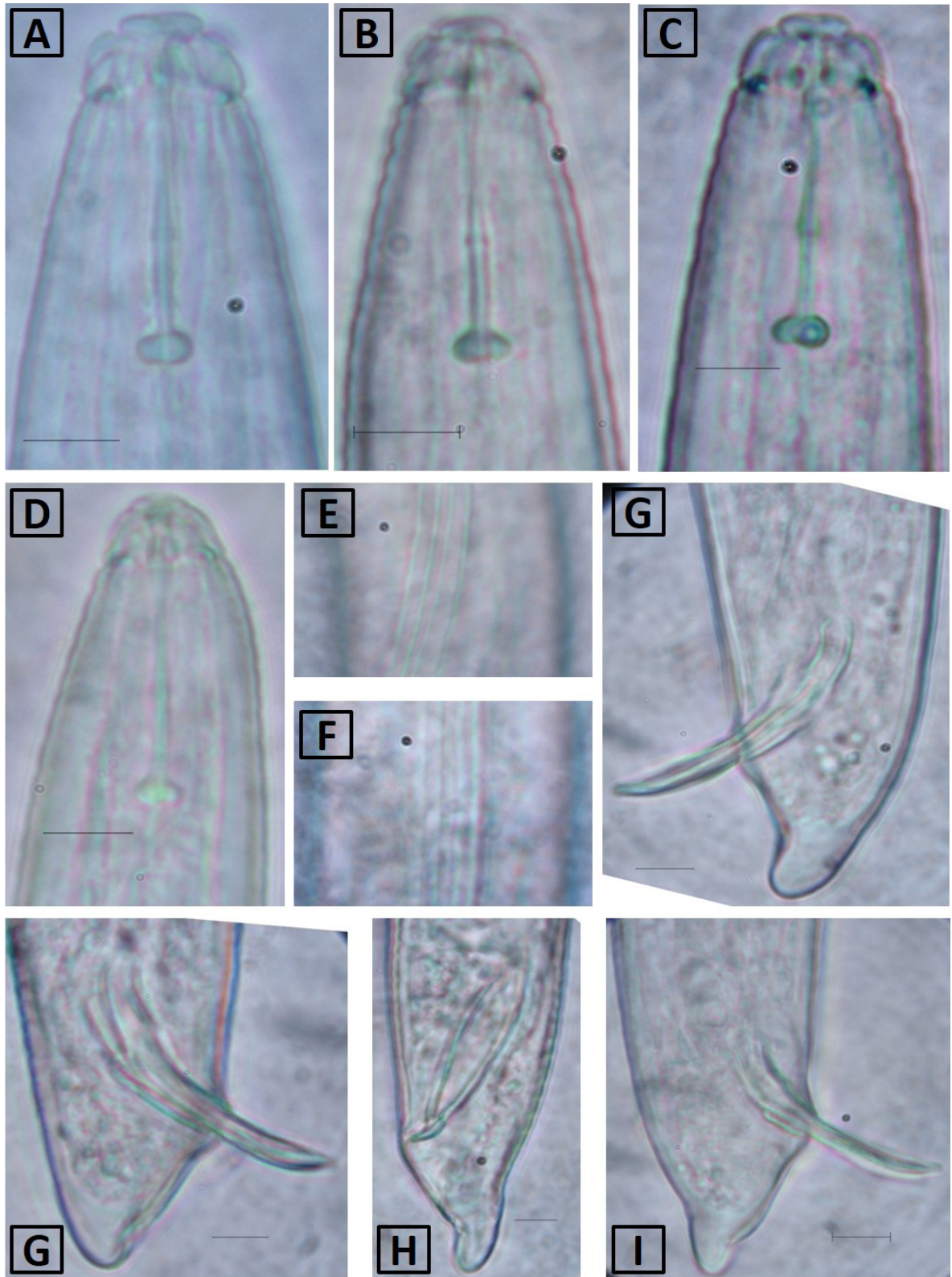


Fig. 3. Variations in anterior end (A-D), posterior end (G-I) and lateral field (E and F) of male. Scale –bar = 5 μm.

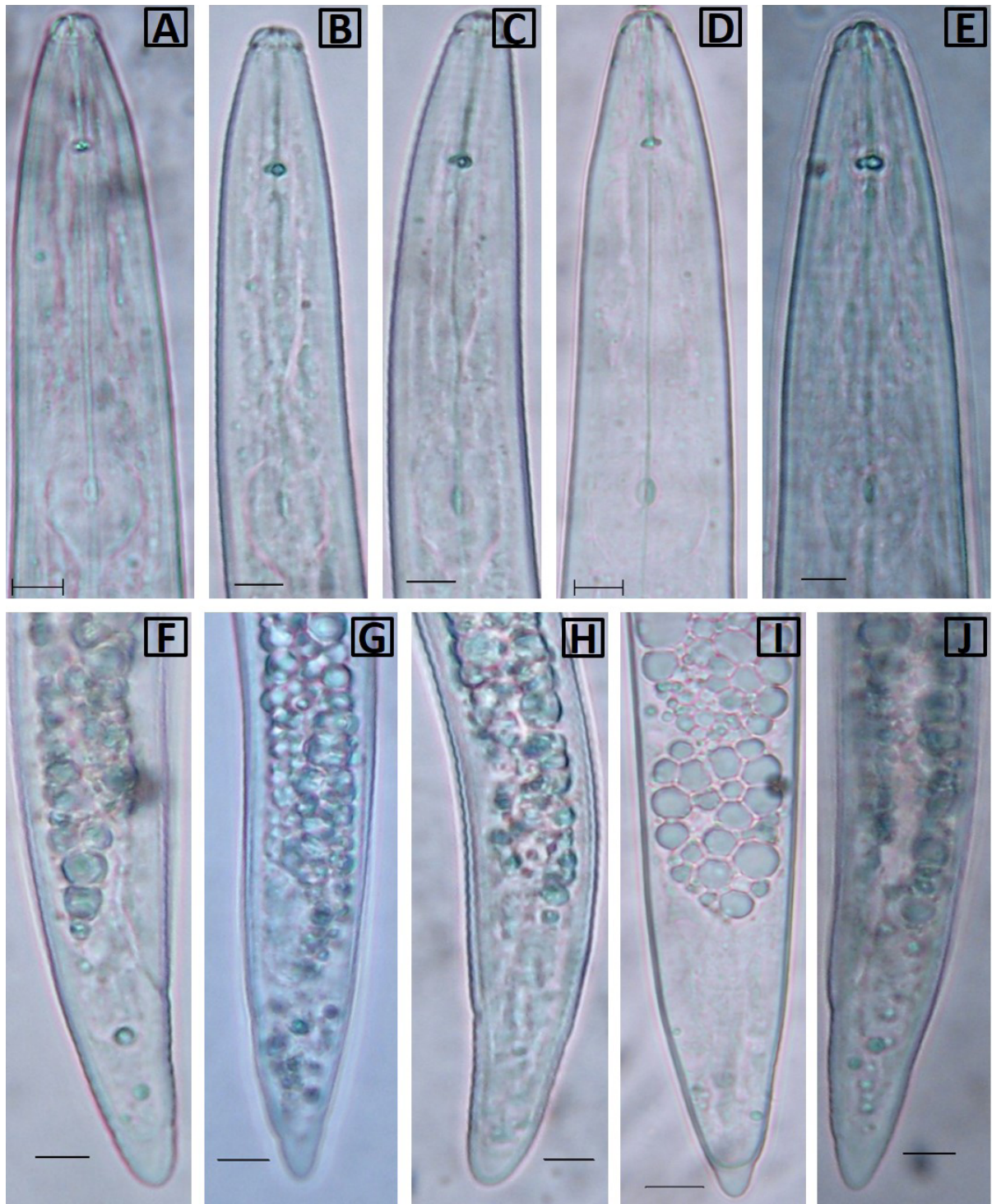


Fig. 4. Variations in anterior end (A-E) and posterior end (F-J) of second stage juveniles. Scale –bar = 5 μ m.

General morphology of M. indica

Female

The body shape appeared pyriform and thick cuticle

with considerable variations in shape and size of the *M. indica* (Fig. 1C). Female with short neck (Fig. 2A, B) with six lips. Posterior part of female generally showed smooth

with protuberance in some specimens. Stylet cone slightly curved dorsally, tapered gradually towards the tip and broadening at the junction with the shaft. Style shaft was cylindrical and broad near the base. Stylet knobs appear transversely ovoid and set off from the shaft. The body dimension showed moderate to high variability ($CV < 20$ to > 20). Mean length of stylet, stylet cone, stylet shaft, length and width of knobs, DGO, length and width of median bulb, length and width of median bulb valve showed little variations. Head to SE-pore showed high variability ($CV \sim 28.02$).

Perineal pattern

The perineal pattern is rounded with rounded dorsal arch (Fig. 2E, F). Lateral field without lateral line or any forking in the striae. Striae were very smooth, continuous and striae forming a distinct tail whorl. Both phasmid and anus were distinct and clearly visible. Morphometric parameters of perineal pattern exhibited moderate variations ($CV < 20$).

Male

The body dimension of male was relatively smaller (Fig. 2C) as compared to *M. incognita*. Head not set off, with two annuli behind head cap. Stylet comparatively thin, stylet cone tip pointed and slightly curved. Stylet knobs slightly set off from shaft with backwardly sloping. Lateral lines 5-6. Tail short conoid with terminal portion narrow with smooth end. The morphometric characters viz., body length, body width, stylet length, stylet cone, stylet shaft, length and width of stylet knobs, DGO, head to metacarpus distance, head to SE-pore position and spicule length showed little difference between two populations (Fig. 3).

Second stage juvenile

The dimension of J2 showed little difference between the populations. Head not set off and head cap slightly outer. Stylet long and knobs rounded to ovoid. Lateral lines four. Length of tail short (Fig. 2D) and hyaline tail terminus very short (Fig. 4), and rectum not inflated. The mean a -, b'' -, b' -, c -, c' -values and mean hyaline tail terminus in both population were almost same.

Egg

Eggs were comparatively long (Fig. 1D); length, width and their ratio varied considerably.

The comparative morphometric analyses of Mehsana (citrus and Bt-cotton) populations of *M. indica*, along with original measurements of Whitehead (1968) from Delhi on citrus is presented in Table I.

Isozyme pattern

The isozyme electrophoretic analysis of young egg laying females of *M. indica* (Mehsana population) was carried out, but despite several attempts the esterase enzyme activity of *M. indica* could not be detected (Fig. 5). Specific esterase phenotypes from other root-knot nematodes, *M. incognita* and *M. javanica* were clearly observed. This result helped to differentiate *M. indica* from common species by the esterase phenotype.

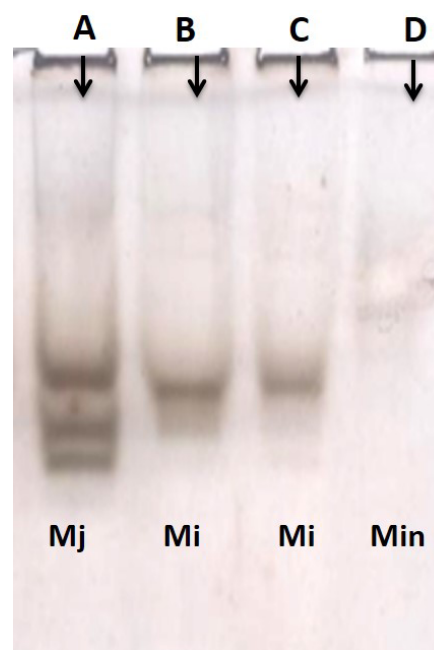


Fig. 5. Beta-esterase phenotypes of *Meloidogyne* species: A, *M. javanica* (as standard); B and C, *M. incognita*; D, *M. indica*.

DISCUSSION

Citrus root-knot nematode (*M. indica*) has been documented in literature as a pest of quarantine significance for its limited distribution in India (Whitehead, 1968; Patel *et al.*, 1999) and in Fujian Province, Wuping of China (Davis and Venette, 2004). The nematode species is known to infect and reproduce on different citrus spp. (Whitehead, 1968; Franklin, 1978; Vovlas and Inserra, 1996, 2000) and morinda (*Morinda officianalis*) (Zhang and Weng, 1991). However, not much information has been generated on diagnostic symptoms, identity of the species, and its host status. The present investigation identified the field infestation of *M. indica* on *Citrus* and Bt-cotton grown in the fields which was previously infested with *M. indica*. Thus, *M. indica* is now distributed in the states of Delhi and Gujarat of India.

Table I.- Comparative morphometric analysis of *M. indica* from citrus and Bt-cotton with Whitehead's citrus population.

Characters	Mehsana population (Bt-cotton)	Mehsana population (citrus)	Delhi population (citrus) (Whitehead, 1968)
Male			
Body length	1140 μ m \pm 171.43 (820-1400)	1266 μ m \pm 99.15 (1130-1400)	--
Body width	25.98 μ m \pm 3.07 (20.90-31.35)	31.73 μ m \pm 4.28 (26.60-35.15)	--
a	44.10 \pm 6.17 (34.21-55.84)	40.47 \pm 5.95 (32.15-46.62)	--
Stylet length	16.58 μ m \pm 0.57 (16.15-18.05)	16.44 μ m \pm 0.64 (15.68-17.10)	--
Stylet cone	9.71 μ m \pm 0.39 (9.50-10.45)	9.31 μ m \pm 0.26 (9.03-9.50)	--
Stylet shaft	5.11 μ m \pm 0.37 (4.75-5.10)	5.32 μ m \pm 0.40 (4.75-5.70)	--
Knobs height	1.76 μ m \pm 0.22 (1.43-1.90)	1.81 μ m \pm 0.21 (1.43-1.90)	--
Knobs width	3.04 μ m \pm 0.47 (1.90-3.80)	2.95 μ m \pm 0.21 (2.85-3.33)	--
DGO	3.25 μ m \pm 0.44 (2.85-3.80)	3.61 μ m \pm 0.54 (2.85-4.28)	--
Head-metacarpus	70.73 μ m \pm 5.55 (58.90-78.85)	76.38 μ m \pm 7.28 (65.55-82.65)	--
b"	16.16 \pm 2.39 (11.51-20.71)	16.69 \pm 1.94 (13.67-18.92)	--
Head-SE pore	108.25 μ m \pm 14.41 (81.70-130.15)	111.91 μ m \pm 16.58 (87.40-126.35)	--
Spicule	25.79 μ m \pm 2.51 (21.85-31.35)	27.36 μ m \pm 1.24 (25.65-28.50)	--
Second stage juvenile			
Body length	462.75 μ m \pm 32.46 (400-535)	474.25 μ m \pm 41.81 (390-525)	414 μ m \pm 4.5 (381-448)
Body width	17.10 μ m \pm 2.97 (13.30-21.85)	18.38 μ m \pm 2.23 (15.20-21.85)	--
Stylet length	13.21 μ m \pm 0.61 (12.35-14.25)	12.33 μ m \pm 0.33 (11.88-13.30)	12 μ m \pm 0.9 (10-14)
MB length	12.45 μ m \pm 0.87 (11.40-14.25)	11.85 μ m \pm 1.11 (10.45-14.25)	--
MB width	8.69 μ m \pm 0.87 (7.60-10.45)	9.17 μ m \pm 0.90 (8.08-10.93)	--
a	27.58 \pm 3.51 (21.97-34.21)	25.99 \pm 2.40 (23.27-30.26)	--
b'	3.68 \pm 0.25 (3.19-4.19)	3.64 \pm 0.33 (3.02-4.13)	--
b"	9.10 \pm 0.68 (7.80-10.24)	9.34 \pm 1.16 (6.73-10.74)	--
c	24.11 \pm 1.26 (21.80-26.61)	35.43 \pm 6.01 (25.66-47.37)	24.9 μ m \pm 1.36 (21.2-31)
c'	1.83 \pm 0.13 (1.54-2.00)	1.45 \pm 0.23 (1.05-1.78)	1.57 μ m \pm 0.012 (1.06-1.78)
Head-SE pore	80.94 μ m \pm 5.24 (73.15-91.20)	79.66 μ m \pm 7.30 (65.55-92.15)	--
DGO	2.83 μ m \pm 0.19 (2.38-3.33)	3.02 μ m \pm 0.32 (2.38-3.80)	--
Head-metacarpus	50.92 μ m \pm 1.93 (44.65-54.15)	51.16 μ m \pm 4.16 (47.50-61.75)	--
Head-oesophageal gland	126.11 μ m \pm 8.10 (114.95-145.35)	130.63 μ m \pm 6.62 (120.65-142.50)	--
Tail length	19.24 μ m \pm 1.60 (17.10-21.85)	13.66 μ m \pm 1.87 (9.50-16.15)	16.8 μ m \pm 1.88 (13-20.1)
Hyaline tail terminus	4.66 μ m \pm 0.40 (3.80-5.70)	4.18 μ m \pm 0.63 (2.85-5.23)	--
Anal body width	10.55 μ m \pm 0.97 (9.50-12.35)	9.52 μ m \pm 0.98 (8.55-11.40)	--
Female			
Body length	683 μ m \pm 138.19 (370-900)	--	--
Body width	418 μ m \pm 70.61 (300-520)	--	--
a	1.64 \pm 0.28 (1.21-2.23)	--	--
Stylet length	13.14 μ m \pm 0.56 (12.35-15.20)	--	14 μ m(12-16)
Stylet cone	8.53 μ m \pm 0.39 (7.60-9.50)	--	--
Stylet shaft	3.90 μ m \pm 0.40 (2.85-4.75)	--	--
Knobs height	1.02 μ m \pm 0.17 (0.95-1.43)	--	--
Knobs width	3.80 μ m \pm 0.15 (3.33-4.28)	--	5 μ m(4-5)
DGO	2.92 μ m \pm 0.32 (2.38-3.80)	--	3 μ m(2-4)
MB length	31.02 μ m \pm 3.10 (25.65-36.10)	--	38 μ m(31-43)
MB width	28.60 μ m \pm 3.93 (23.75-36.10)	--	39 μ m(33-46)
Length MB valve	12.59 μ m \pm 0.86 (11.40-15.20)	--	14 μ m(13-16)
Width MB valve	8.41 μ m \pm 0.89 (6.65-9.50)	--	9 μ m(7-11)
Head-SE pore distance	22.71 μ m \pm 6.36 (14.25-36.10)	--	--

Characters	Mehsana population (Bt-cotton)	Mehsana population (citrus)	Delhi population (citrus) (Whitehead, 1968)
Egg			
Length	114.75µm±3.53 (110-120)	--	77µm±4.70 (381-448)
Width	44.88µm±3.58 (40-50)	--	30µm±1.90 (26.35)
Ratio	2.57±0.20 (2.20-2.88)	--	--
Perineal pattern			
Vulval slit length	21.00µm±2.95 (13.30-25.65)	--	--
Inter-phasmid	24.18µm±3.56 (17.10-30.40)	--	--
Vulva-anus	21.80µm±3.56 (14.25-27.55)	--	--
Vulval slit-tail terminus	31.07µm±4.04 (23.75-38.95)	--	--

Efforts for testing crop hosts viz., tomato (cv Rutgers), brinjal (cv local), and citrus (kazgi lime, cv local) against *M. indica* with the soil infested with the same species, indicated that *M. indica* can infect only citrus, though the soil from both Bt-cotton and citrus rhizosphere are co-inhabited by the *M. indica* and *M. incognita*. None of the citrus and Bt-cotton plants were found infected by *M. incognita* either in field or pot conditions. The galls produced on the citrus root appeared thick with small eggmass (less than 50 eggs) concealed inside the root. The species was first time reported from Delhi on citrus and further occurrence or interception was not noted earlier from India reveals its limited distribution. Further occurrence of *M. indica* on citrus (cv kazgi lime) and Bt-cotton from Mehsana district of Gujarat are believed to be the recent report from additional areas of infestation within India. Whitehead (1968) described the species based on limited number of specimens, and adequate morphometric data was not presented. The present study generated detailed morphology and morphometric illustrations of *M. indica* populations from Mehsana (citrus and Bt-cotton). These are in conformity with the description of Whitehead (1968) with slight deviations. The dimension of median bulb in female was smaller in the Bt-cotton of Mehsana population. Body dimension, stylet cone, shaft and other parameters of mature female were not taken into consideration in the previous studies. The length of second stage juvenile, tail length and egg dimensions of *M. indica* were relatively larger than that of Whitehead's Delhi (citrus) population. However, these differences may be considered as intra-specific variation for the species. The second stage juveniles of *M. indica* showed distinctiveness in anterior end as well as posterior end. The tail shape observed as typically short conoid with blunt terminus and smooth and hyaline tail region. Further this study provided differential and supplementary taxonomic characters of male, female, second stage juvenile and eggs. The perineal pattern morphology was typical for species as described by Whitehead (1968). The

morphometrics of female from citrus was not included here as sufficient number of specimens was not available for lateral mounting and measurements. However, male and second stage juveniles of *M. indica* were studied for morphometric variations. Morphometrics of male were not studied earlier and, therefore, the data generated here could not be compared with Whitehead's population. In the original description, only three males were available for morphological description, they are in conformity with Bt-cotton and citrus population. However, the number of lateral lines in the lateral fields noted here varied from five to six instead of four in the original description. The tail shape and position of cloaca was almost similar with that of Whitehead's descriptions. The second stage juvenile of *M. indica* showed distinctiveness in anterior as well as posterior ends, tail shape typically short conoid, terminus blunt and smooth, and hyaline tail region. The tail shape and position of cloaca are almost similar with that of Whitehead's descriptions. The scanning electron microscopy (SEM) photomicrograph also showed minute morphological details of male, female and juveniles.

This study provides morphological, morphometric and biochemical information on *M. indica* infecting citrus and Bt-cotton in India. The observation on light microscopy, host tests, beta-esterase enzyme phenotypes are quite relevant to *M. indica* for better understanding of the species described by Whitehead (1968) from India.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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